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*** YOU HAVE NEW MAIL ***

=> s primer (2a) biotinylated
L1 908 PRIMER (2A) BIOTINYLATED

=> s primer (a) biotinylated
L2 594 PRIMER (A) BIOTINYLATED

=> s l2 and dye labeled ribonucleotide?
L3 0 L2 AND DYE LABELED RIBONUCLEOTIDE?

=> s l1 and label? ribonucleotide
L4 4 L1 AND LABEL? RIBONUCLEOTIDE

=> d l4 bib abs 1-4

L4 ANSWER 1 OF 4 MEDLINE
AN 96229026 MEDLINE
DN 96229026 PubMed ID: 8849029
TI Time-resolved fluorometric hybridization assays with RNA probes
synthesized from polymerase chain reaction-generated DNA templates.
AU Radovich P; Bortolin S; Christopoulos T K
CS Department of Chemistry and Biochemistry, University of Windsor, Ontario,
Canada.
SO ANALYTICAL CHEMISTRY, (1995 Aug 1) 67 (15) 2644-9.
Journal code: 0370536. ISSN: 0003-2700.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199610
ED Entered STN: 19961106
Last Updated on STN: 19961106
Entered Medline: 19961021
AB DNA templates suitable for direct synthesis of RNA probes are produced by
the polymerase chain reaction. The nucleic acid sequence of interest is
amplified using a downstream primer carrying the T7 RNA polymerase
promoter sequence. The modified primer is incorporated into the amplified

DNA, which is subsequently used for RNA probe synthesis in the presence of T7 RNA polymerase and a **hapten-labeled ribonucleotide** (digoxigenin-UTP). As a model, we prepared RNA probes specific for the BCR-ABL mRNA characteristic of chronic myelogenous leukemia. The probes are used in time-resolved fluorometric hybridization assays. Mixtures of BCR-ABL positive and negative cells, as well as whole blood samples, are analyzed. The sample mRNA is amplified using a **biotinylated upstream primer**. The amplified product (target DNA) is captured onto streptavidin-coated wells and hybridized to the RNA probe. The hybrids are detected with an alkaline phosphatase (ALP)-labeled antibody. ALP hydrolyzes the phosphate ester of fluorosalicylic acid, and the fluorosalicylate produced forms highly fluorescent ternary complexes with Tb(3+)-EDTA, which can be quantified by measuring the Tb3+ fluorescence in a time-resolved mode. As low as 0.4 fmol of target DNA can be detected. Also, a single leukemic cell may be detected in the presence of 0.5 million "normal" cells.

L4 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS
 AN 1995:653000 CAPLUS
 DN 123:75783
 TI Time-Resolved Fluorometric Hybridization Assays with RNA Probes
 Synthesized from Polymerase Chain Reaction-Generated DNA Templates
 AU Radovich, Paula; Bortolin, Susan; Christopoulos, Theodore K.
 CS Department of Chemistry and Biochemistry, University of Windsor, Windsor,
 ON, N9B 3P4, Can.
 SO Analytical Chemistry (1995), 67(15), 2644-9
 CODEN: ANCHAM; ISSN: 0003-2700
 PB American Chemical Society
 DT Journal
 LA English
 AB DNA templates suitable for direct synthesis of RNA probes are produced by the polymerase chain reaction. The nucleic acid sequence of interest is amplified using a downstream primer carrying the T7 RNA polymerase promoter sequence. The modified primer is incorporated into the amplified DNA, which is subsequently used for RNA probe synthesis in the presence of T7 RNA polymerase and a **hapten-labeled ribonucleotide** (digoxigenin-UTP). As a model, we prep'd. RNA probes specific for the BCR-ABL mRNA characteristic of chronic myelogenous leukemia. The probes are used in time-resolved fluorometric hybridization assays. Mixts. of BCR-ABL pos. and neg. cells, as well as whole blood samples, are analyzed. The sample mRNA is amplified using a **biotinylated upstream primer**. The amplified product (target DNA) is captured onto streptavidin-coated wells and hybridized to the RNA probe. The hybrids are detected with an alk. phosphatase (ALP)-labeled antibody. ALP hydrolyzes the phosphate ester of fluorosalicylic acid, and the fluorosalicylate produced forms highly fluorescent ternary complexes with Tb3+-EDTA, which can be quantified by measuring the Tb3+ fluorescence in a time-resolved mode. As low as 0.4 fmol of target DNA can be detected. Also, a single leukemic cell may be detected in the presence of 0.5 million "normal" cells.

L4 ANSWER 3 OF 4 USPATFULL
 AN 2003:17337 USPATFULL
 TI **Dye-labeled ribonucleotide** triphosphates
 IN Fisher, Peter Virgil, El Granada, CA, UNITED STATES
 Vatta, Paolo, San Mateo, CA, UNITED STATES
 Khan, Shaheer H., Foster City, CA, UNITED STATES
 PI US 2003013089 A1 20030116
 AI US 2001-886011 A1 20010622 (9)
 DT Utility
 FS APPLICATION
 LREP FINNEGAN, HENDERSON, FARABOW, GARRETT &, DUNNER LLP, 1300 I STREET, NW,

09567863

WASHINGTON, DC, 20006
CLMN Number of Claims: 123
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 2302

AB The invention provides novel dye-**labeled** **ribonucleotide** analogs and methods for synthesizing those analogs. The compounds of the invention are especially useful for DNA sequencing by the polymerase chain reaction.

L4 ANSWER 4 OF 4 USPATFULL
AN 2002:346801 USPATFULL
TI Method for identifying polymorphisms
IN Stanton, Jr., Vince P., Belmont, MA, United States
Wolfe, Jia Liu, Winchester, MA, United States
Kawate, Tomohiko, Cambridge, MA, United States
Verdine, Gregory L., Cambridge, MA, United States
Olson, Jeffrey, Chelmsford, MA, United States
PA Variagenics, Inc., Cambridge, MA, United States (U.S. corporation)
PI US 6500650 B1 20021231
AI US 2000-655104 20000905 (9)
RLI Continuation-in-part of Ser. No. US 1999-394467, filed on 10 Sep 1999
Continuation-in-part of Ser. No. US 1999-394457, filed on 10 Sep 1999
Continuation-in-part of Ser. No. US 1999-394774, filed on 10 Sep 1999
Continuation-in-part of Ser. No. US 1999-394387, filed on 10 Sep 1999
PRAI US 1998-102724P 19981001 (60)
US 1999-149533P 19990817 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Riley, Jezia
LREP Lyon & Lyon LLP
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 72 Drawing Figure(s); 59 Drawing Page(s)
LN.CNT 6037
AB The present invention relates to methods for the detection of polymorphism in polynucleotides by using hybridization of fragments of segments of a polynucleotide suspected of containing a polymorphism with an oligonucleotide having a sequence complementary to a fragment identifying the polymorphism and subsequent detection of incorporated labels in the oligonucleotide-fragment duplex.

=> d 14 4 kwic

L4 ANSWER 4 OF 4 USPATFULL
SUMM In further aspect of this invention, the detectably labeled nucleotide comprises a detectably **labeled ribonucleotide**.
SUMM In another aspect of this invention, the detectably **labeled ribonucleotide** comprises a radioactive ribonucleotide or a ribonucleotide containing a fluorophore.
DETD . . . point, a detectable label is incorporated into the system, either by use of a labeled primer, a labeled nucleotide, a **labeled ribonucleotide**, a labeled, modified nucleotide or a labeled, modified ribonucleotide. Furthermore, a label may be incorporated during the cleavage reaction using . . .
DETD . . . above, this can be accomplished by using labeled TCEP or a secondary amine during the cleavage reaction or using a **labeled ribonucleotide** during PCR amplification.
DETD . . . hair-pin loop structures bring in close proximity the

incorporated fluorescent label at the 3' end (either via incorporation of a **labeled ribonucleotide** or by a labeled TCEP or secondary amine) and the 5' fluorescent label attached to the primer. For signal quenching. . .

DETD 3. An alternative to the above methods includes using two different primers. As in the previous two methods, either a **labeled ribonucleotide** incorporated during PCR or a labeled TCEP or secondary amine incorporated during the chemical cleavage reaction would be used to. . .

DETD . . . are subjected to chemical cleavage. As previously described, the cleavage may include labeled TCEP or labeled secondary amine or a **labeled ribonucleotide** or modified ribonucleotide may be used during the PCR amplification reaction.

DETD . . . purified using streptavidin agarose and the non-biotinylated strand from each PCR product was eluted and used as a template for **primer extension**. The **biotinylated primer** RFC bio was extended on these templates in the presence of dATP, dCPT, dTTP and 7-methyl dGTP. The extended products. . .

DETD . . . the following 10-mers depending on the reverse primer used in the PCR reaction, RFC (3054.9) or RFC mut (3039.88). The **biotinylated 20-mer primer** is also present because it was provided in excess in the extension reaction. The 10-mer fragments for RFC and RFC. . .

DETD FIG. 4 is the RFC mass spectrogram of the RFC sample. The peak on the far right is the **biotinylated primer** band that was used as a standard to calculate the molecular weights of all other bands. The left side of. . .

CLM What is claimed is:

11. The method of claim 9, wherein the detectably labeled nucleotide comprises a detectably **labeled ribonucleotide**.

12. The method of claim 11, wherein the detectably **labeled ribonucleotide** comprises a radioactive ribonucleotide or a ribonucleotide containing a fluorophore.

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